

REMARKS

Claims 20, 23, and 26 were under examination in the outstanding Office Action (hereinafter, "Action") dated May 16, 2006. Claims 1-19, 21, 22, 24, and 25 are pending but withdrawn from consideration as being drawn to a non-elected invention. Claims 20 and 23 are amended herein for clarity and to more particularly define the invention. In addition, withdrawn claim 1 has been amended herein. Support for these amendments is found throughout the specification. No new matter is added by these amendments and their entry and examination are respectfully requested. In light of these amendments and the following remarks, applicants respectfully request reconsideration of this application and allowance of the pending claims to issue.

The issues raised in the Office Action are addressed below in the order in which they were raised.

I. Objections to the Specification.

The disclosure was objected to for containing embedded hyperlinks. Action, page 3. The disclosure has been amended to delete the embedded hyperlinks on pages 12 and 13. Applicants note that no hyperlink was found on page 19, line 19, of the specification as indicated in the Action. A search of the text did not reveal any additional hyperlinks to those identified by the Action at pages 12 and 13 of the specification. Applicants request that the Examiner please contact Applicants' representative if this is mistaken so that proper correction can be made.

The disclosure was also objected to for failure to refer to sequences by sequence identifiers. Applicants have amended the disclosure to include sequence identifiers and have submitted a sequence listing in both paper and computer readable format.

Accordingly, Applicants respectfully submit that these objections have been obviated and respectfully request that they be withdrawn.

II. Statement In Support of Filing A Sequence Listing Under 37 CFR § 1.821(F).

Submitted herewith is a copy of the Sequence Listing for the above-identified patent application in computer readable form and in paper copy. I hereby state that the content of the paper and computer readable copy of the Sequence listing is the same. I also hereby state as required under 37 CFR § 1.821(h) that the computer readable copy and the paper copy of the sequence listing submitted concurrently herewith contains no new matter, nor does it go beyond the disclosure of the application as filed.

III. Rejection under 35 U.S.C. § 112, first paragraph, enablement.

Claims 20, 23, and 26 stand rejected under 35 U.S.C. § 112, first paragraph, for allegedly lacking enablement. Action, page 4. Specifically, the Action states that the specification "does not reasonably provide enablement for a plant cell or plant transformed with any RNA precursor construct operably linked to a promoter comprising any miRNA sequence that is complementary to a portion of target sequence of interest." Action, page 4.

Claim 20 is amended herein to recite a plant stably transformed with an miRNA precursor construct, said miRNA precursor construct comprising a first promoter that drives expression in a plant cell operably linked to a first nucleotide sequence encoding an miRNA precursor, said precursor having at least one miRNA sequence incorporated into the miRNA precursor, wherein said miRNA sequence is complementary to a portion of a first target sequence.

Additionally, claim 23 is amended herein to recite a plant cell stably transformed with an miRNA precursor construct, said miRNA precursor construct comprising a first promoter that drives expression in a plant cell operably linked to a first nucleotide sequence encoding an miRNA precursor, said precursor having at least one miRNA sequence incorporated into the miRNA precursor, wherein said miRNA sequence is complementary to a portion of said first target sequence.

Thus, claims 20 and 23 now recite an "miRNA precursor construct" or "miRNA precursor" rather than "RNA precursor construct" or "precursor RNA", respectively. Support for these amendments is found throughout the specification as originally

filed, for example, at least page 9, lines 26-30, page 10, lines 12-13, and page 31, line 20, through page 35, line 14.

Applicants respectfully submit that the presently claimed invention is enabled for a wide variety of miRNA precursors, in addition to the miRNA 167 and miRNA 171 precursors disclosed in Examples 3 through 5. As an initial point, Applicants note that plant miRNA precursors were well-known in the art prior to the filing date of the present application, July 21, 2003 (priority date of July 19, 2002). *See*, for example, Reinhart et al. (Genes & Development 16:1616-1626 (2002)) and Llave et al. (Plant Cell 14: 1605-1619 (2002)) (copies submitted with IDS dated October 23, 2003). In addition, numerous other publications describe what constitutes an miRNA precursor and how these precursors are identified. *See*, for example, Bartel et al. (Plant Physiol. 132: 709-717 (2003)), and Kidner et al. (Trends in Genet. 19:13-16 (2003)) (copies enclosed herewith). These publications and others show that miRNA precursors all share structural features in common that identify them as miRNA precursors. The common features of the miRNA precursors are what direct the processing of the mature miRNA from the miRNA precursor by the activity of the DICER-LIKE1 enzyme. Therefore, based on the commonly shared features of miRNA precursors and the teachings of the present specification, one of skill in the art would expect that a wide variety of miRNA precursors could be used as a backbone to produce a functional miRNA via the miRNA biogenesis pathway.

In the present application, Example 3 of the specification provides detailed methods for making miRNAs and miRNA precursor constructs using a strategy wherein the miRNA precursor constructs mimic any of the many known endogenous miRNA precursors. The specification teaches the structural features of miRNA precursors that are important to include in order to practice the invention. On page 32, second paragraph, the specification teaches that miRNA precursors "are characteristically stem-loop structures in which the stem is not completely double-stranded." Furthermore, the specification teaches that "the single-stranded miRNA is processed from the stem of the precursor and is commonly flanked by bulges" and that the "designer miRNA precursor will contain a loop chosen from an endogenous miRNA precursor." *Id.* Finally, the specification teaches that the sequence of the

designer miRNA is inserted in a position analogous to the authentic miRNA within the precursor and features such as bulges are included from the context of the endogenous precursor. *Id.* Specific non-limiting examples of how these teachings can be used are presented in the Example section of the specification where designer miRNA precursors are constructed mimicking the miRNA 167 and miRNA 171 precursors.

Additionally, it was known in the art prior to the filing of the present application that any particular native miRNA sequence can have more than one precursor miRNA. For example, Reinhardt et al. (Genes & Development 16:1616-1626 (2002)) shows that for miR156 there are 6 different miRNA precursor sequences. (See, Figure 1, page 1619). Each of these miRNA 156 precursors is processed by an enzyme called DICER-LIKE1 to produce the same small RNA, miRNA 156. Similarly, for miRNA 167, there are four different loci that produce the miRNA precursors, which are then processed by DICER-LIKE1 to produce the small RNA, miRNA 167. Thus, the term miRNA 167 precursor, in itself, refers to four different precursors that share in common only the mature miRNA 167 sequence.

Therefore, using the known, commonly shared, structural features of endogenous miRNA precursors as taught in the present specification, one skilled in the art is able to practice the present invention with a multiplicity of miRNA precursors as backbones for the production of functional miRNAs without undue experimentation. Further, since the specification teaches how to design and use miRNA precursors generally, limiting the class of miRNA precursors of the present application to the miRNA167 precursor would unduly limit the Applicants' patent rights.

Finally, as expected, based on the structural features shared by all miRNA precursors, the present invention has been shown to work as described using a variety of different miRNA precursors. For example, Vaucheret et al. demonstrated that the precursor for miRNA 168 could be modified to target a particular sequence (Vaucheret et al., Genes & Development 18: 1187-1197 (2004); copy enclosed). Schwab et al. (Plant Cell 18: 1121-1133 (2006); copy enclosed) demonstrated in a more global way that the invention works as described in the present application.

These investigators used two different miRNA precursors, miRNA172a and miRNA319a, as backbones to produce designer miRNAs to target specific genes. The investigators reported that their designer miRNA precursors worked well in this capacity.

Thus, the foregoing discussion demonstrates that the Applicants' specification, as originally filed, enables one of skill in the art to use a multiplicity of miRNA precursors as backbones to produce functional miRNAs via the miRNA biogenesis pathway that can be used to stably transform a plant or plant cell. Accordingly, Applicants respectfully submit that the presently claimed invention is enabled and respectfully request the rejection of claims 20, 23, and 26 for lack of enablement be withdrawn.

IV. Rejection under 35 U.S.C. § 112, first paragraph, written description.

Claims 20, 23, and 26 stand rejected under 35 U.S.C. § 112, first paragraph, for allegedly failing to comply with the written description requirement. Action, page 7.

As discussed above, claims 20 and 23 are amended herein to recite an miRNA precursor construct or miRNA precursor rather than RNA precursor construct or precursor RNA, respectively. Applicants respectfully submit that the concerns of the Examiner are addressed by these amendments along with the foregoing discussion and respectfully request withdrawal of the rejection of claims 20, 23, and 26 under 35 U.S.C. § 112, first paragraph.

V. Rejection under 35 U.S.C. § 102(b) and § 103(a).

Claims 20, 23, and 26 stand rejected under 35 U.S.C. § 102(b) as allegedly anticipated by, or in the alternative under 35 U.S.C. § 103(a) as allegedly obvious over Chuang et al. (PNAS 97:4985-4990 (2000)). Action, page 9. Specifically, the Action states that Chuang et al. teaches a plant or plant cell transformed with a construct comprising a double stranded RNA-expressing construct corresponding to a gene AGAMOUS (AG), which is involved in flower development. *Id.* The Action further states that the stable introduction of the construct into the genome of the

transgenic plant cell or plant resulted in heritable down-regulation of endogenous AG mRNA, suggesting that endogenous mRNA is the target of double stranded-mediated (RNA precursor) genetic interference. *Id.*

As previously discussed, claims 20 and 23 are amended herein to recite an miRNA precursor construct or miRNA precursor rather than RNA precursor construct or precursor RNA, respectively. Chuang et al. does not teach or suggest miRNA or miRNA precursors as recited in the presently claimed invention.

As the Action states, Chuang et al. discusses the use of fully double stranded RNA (dsRNA) for gene silencing. Action, page 9-10. The differences between dsRNA and miRNA precursors were well known at the time of filing the present invention. See, for example, Bartel et al. (2003) and Kidner et al. (2003). Those of ordinary skill in the art would understand, for example, that miRNA precursors are only partially double stranded stems with characteristic loops and bulges whereas dsRNA is fully double stranded. Further, the processing of miRNA precursors produces a single small RNA (miRNA). In contrast, fully dsRNA is processed into a population of siRNAs that represents the entire dsRNA trigger. Additionally, different DICER-LIKE enzymes are required for the production of miRNAs versus siRNAs. (See, Reinhardt et al. (2002) and Finnegan et al. Current Biol. 13:236-240 (2003) (copy enclosed herewith)). Another difference concerns the differential regulation of the miRNA and siRNA pathways with respect to the viral suppressor of silencing called HC-Pro. Mallory et al. (PNAS 99: 15228-15233 (2002)) (copy enclosed herewith) has shown that HC-Pro allows enhanced accumulation of all endogenous miRNAs examined, but blocks the accumulation of the siRNAs that mediate sense-transgene silencing. Thus, miRNA precursors and dsRNA are not only structurally and genetically different but are also regulated in ways that are distinctly different.

Accordingly, Applicants respectfully submit that the presently claimed invention is both novel and non-obvious over Chuang et al. and respectfully request that the rejections of claims 20, 23 and 26 under 35 U.S.C. § 102(b), or in the alternative, under 35 U.S.C. § 103(a) over Chuang et al. be withdrawn.

Conclusion.

The points and concerns raised by the Examiner having been addressed in full, it is respectfully submitted that this application is in condition for allowance, which action is respectfully requested. Should there be any remaining concerns, the Examiner is encouraged to contact the undersigned attorney to expedite the prosecution of this application.

No fee is believed due. However, the Commissioner is hereby authorized to charge any deficiency, or credit any refund to our Deposit Account No. 50-0220.

Respectfully submitted,



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